

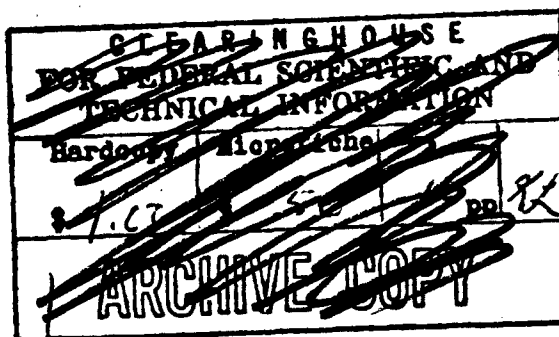
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THEIR APPLICATION IN MICROBIOLOGY

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## PROPERTIES OF ANTIBODIES FIXED ON PARTICLES AND THE PERSPECTIVE OF THEIR APPLICATION IN MICROBIOLOGY

[Following is the translation of an article by A. K. Adamov, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 11, 1964, pages 3--7. The article was submitted to the editors on 14 Dec 1963. Translation performed by Sp/4 Richard M. Koplen]

The properties of particles and the properties of immune sera influence the sensitivity and specificity of antibody--particle complexes (suspension antibodies) (Adamov, 1963). Substances having particles which, following combining with antibodies, preserve their stability in an 0.85% solution of sodium chloride and in solutions of heterologous antigens are suitable for the synthesis of suspension antibodies. The stability of particles of organic substances depends on their chemical structure. Among the organic substances, the molecules of which contain condensation benzene rings, derivatives which form stable suspension are encountered more frequently. Quinone and hydroxyl groups, and also the situation of hydroxyl groups in the rings of molecules (Adamov and Berdnikov, 1963), influence the stability of particles in a number of anthracene derivatives.

The immunological activity of antibody -- particle complexes apparently depends on the degree of stability of the particles, however, it is necessary to confirm this position with experimental investigations. Stable antibody-particle complexes are obtained from powders of alizarin, anthragallol, indanthrene, 2-methyl-1,4-naphthaquinone, pigment of green-blue phthalocyanine, bismuth-orsinol, bismuth-pyrocatechol, diatomaceous earth, animal charcoal, etc. Antibodies fixed on particles of alizarin and anthragallol have the highest immunological activity and specificity.

Sera used for preparation of suspension antibodies are obtained by a specially developed method of immunization. During synthesis of suspension antibodies against microbes of the enteric group, the best results are attained with sera prepared by the following method:

Rabbits are immunized intravenously with formolvaccine containing 5 billion cells. According to this plan, 1 ml. is administered the first time and the 2nd, 3rd, and 4th times, 2 ml. with 5 day intervals between. On the 7th day after the 4th injection, the rabbits were exsanguinated. In the event it is not possible to use formolvaccine, at first the vaccine is administered separately and then a 0.5% solution of formalin in an 0.85% solution of sodium chloride based on the method indicated above. Here, agglutinating sera with a titer of 1:12,800--1:25,600 are obtained. The rabbits whose sera were to be used for the preparation of normal suspension globulins received an 0.5% solution of formalin in an 0.85% solution of sodium chloride in the same quantities as the vaccine.

On administration of formalin, the organism produces antibodies possessing a high immunological activity after adsorption on particles, and retaining specific properties for a long time (over 12 months).

The titer of antibodies in the sera, the composition of protein fractions, and filtrations of sera through asbestos filters also influence the sensitivity and specificity of suspension antibodies. Antibodies adsorbed on particles from sera with a 1:1600-1:3200 titer (titers after sorption with heterologous antigens) possessed the maximum immunological activity. During the use of sera with higher titers, the sensitivity of suspension antibodies prepared from them did not increase.

The mechanism of filtration influence of sera on the immunological activity of suspension antibodies obtained from them was not determined. It has been reported that filters adsorb nonimmune proteins from sera and possibly disrupt the bond of antibody molecules with molecules of nonimmune proteins of the sera. From sera filtered through asbestos filters, suspension antibodies were obtained having 10-20 times superior activity than suspension antibodies prepared from nonfiltered sera.

Protein fractions hindering the synthesis of suspension antibodies are removed from sera by the method of sorption on heterologous antigens.

Sorption of antisalmonella, antidyenteric, and antitubulin sera is caused by heterologous antigens based on the following scheme: To serum diluted with a 1:5 solution containing 0.85% sodium chloride and 1% boric acid, antigens are added on the basis of 900 billion cells of enteric bacteria to 25 ml of diluted serum; the mixture is kept for 1 hour at 37° and then is centrifuged and the supernatant serum filtered through the SF (GOST 480-41) asbestos plate with a diameter

of 35 mm; to the filtered serum, 300 billion anthracoid cells are added; they are kept for 18 hours at 6-8° and are again filtered through the asbestos plate. If large volumes of sera are prepared, then filtration is carried out with portions of 25 ml. of serum through one asbestos plate. For obtaining monoreceptive sera, the necessary antigens are used, correspondingly decreasing the number of cells of enteric bacteria.

For preparation of suspension antibodies against plague and brucellosis microbes, special schemes for the exhaustion of immune sera are used. The serum, after exhaustion, is filtered only once through an OST 10931-40 asbestos plate with a diameter of 35 mm (other filters did not have the same influence). Suspension antibodies prepared from sera with a 1:400 titer of agglutinins and higher (Adamov with coauthors, 1962; Adamov and Karpuzidi, 1963) possess a high immunological activity. Yafayev (1963) obtained active suspension antibodies by sorption of antibodies on particles of carbon from immune gamma-globulin.

During sorption of antibodies on 1-2 microbial particles of certain substances (alizarin and others), stable complexes of an antibody-particle are formed which are 1-2 mk. in size (fig. 1) and conglomerations of complexes, reaching dimensions of 3-12 mk. (fig. 2). The combining of the properties of particles and the properties of immune antibodies in suspension antibodies causes the appearance of new qualities -- a high sensitivity and specificity.

The reason for the increase in sensitivity of the antigen-antibody reaction, when using antibodies which were fixed in particles, are determined by the sizes of antibody-particle complexes. Suspension antibodies exceed the dimensions of antibody molecules by approximately 30 million times. During the introduction of large particles of suspension antibodies in the reaction, one must expect, proceeding from theoretical calculations, increases of reaction sensitivity by many times, because for calculation of results, it is necessary for a drop to contain no more than 100-200 specific agglomerates 0.25 mm in size. For formation of this quantity of agglomerate, around 100-200 thousand particles of suspension antibodies and an equal quantity of microbial cells are necessary. However, in the experiments visible agglomerates were formed by the interaction of 50 million particles of suspension antibodies and no less than 1 million microbial cells. Contradiction between calculations and experimental data testifies to the necessity for further study of the properties of suspension antibodies, and also to the possibility of a considerable increase in

sensitivity of the agglomeration reaction of suspension antibodies.

Suspension antibodies are more specific than molecular ones (antibodies contained in immune sera). Application of antiplague suspension antibodies (see table), for the first time, made it possible to distinguish plague causative agents, based on the antigenic structure, from the bacteria of pseudotuberculosis of rodents (Adamov with co-authors, 1962).

With the aid of suspension antibodies, it is possible to differentiate botulism causative agents of A and B types from sporogenic bacilli which are related to them, based on antigenic structure (Adamov and Bulatova, 1963). Suspension antibodies are suitable for identification of pathogenic microbes, spontaneously agglutinating in serum or in a physiological solution, and also the R-forms of pathogenic microbes (with the exception of variants, containing absolutely no specific substances).

The increase of specificity of the immunological reaction of the antigen-antibody when using suspension antibodies is explained by 3 basic peculiarities of suspension antibodies.

The first peculiarity of suspension antibodies lies in the fact that they enter into the reaction of agglomeration only with strictly specific antigens. Evidently, they combine also with related antigens, but agglomerations of suspension agglutinins do not occur here. Especially demonstrative is the phenomena observed in the agglomeration reaction of suspension agglutinins with plague microbes. The latter synthesize the capsular antigen slowly at 28° and after a 2-day incubation it is possible, with the aid of luminescent antibodies, to detect only a thin capsule in the plague microbes. However, microbes from such a culture are not able to cause agglomeration of homologous suspension agglutinins. Only after the capsular antigen, under definite conditions, acquires complete conformity to the homologous antigen does it cause the specific agglomeration of suspension antibodies. The data presented, and also experiments with salmonella, showed that alizarin suspension antibodies are suitable for a determination of the quality of antigens and can be used in vaccine production for control of the antigenic composition of vaccines.

The second peculiarity of suspension antibodies is caused by their dimensions. Unlike molecular antibodies, the interaction of the antigen occurs on the surface of the suspension antibody. The antigen reacts with the antibody, only to a limited sector, and a large portion of it remains free. The antibody which is fixed on the particle also reacts with the antigen on a limited sector, since a large portion of its surface is covered with neighboring antibodies and particles. In

connection with this, in the reaction with suspension antibodies, it is not possible for a continuous film to form either around molecules of antigens or around molecules of antibodies. In the agglutination reaction on the surface of cells, a continuous film from antibody molecules forms which makes the cells unstable and causes their agglutination into agglutinates. A film from antibodies also forms on the surface of cells containing related antigens. In this case, para-agglutination occurs. Para-agglutination does not occur with suspension antibodies.

The third peculiarity of suspension antibodies lies in the unique physical interaction of suspension antibodies and suspension globulins (normal globulins which were adsorbed on particles of suspension) with suspensions of certain strains of saprophytic microbes. A number of strains (including certain para-agglutinating strains), not containing antigens homologous to antibodies which were fixed on particles, cause agglomeration of suspension antibodies and of normal suspension globulins. Certain strains of enteric bacteria (7-10% from freshly isolated strains), staphylococci, causative agents of rodent pseudotuberculosis, anthracoids, and other species of microbes cause nonspecific reactions of a similar type. Evidently, cells of the strains indicated above, in the form of microbes, possess a weak electric charge and during mixing with suspended antibodies, a neutralization of the charge takes place which is accompanied by a reciprocal precipitation of suspensions with the formation of nonspecific agglomerates. Nonspecific reactions are removed after treatment (sorption) of microbial suspensions with trisubstituted calcium phosphate (Adamov, 1959).

Physical properties of microbial cells may change under the influence of certain components of the nutrient media. Microbial suspensions which were prepared from cultures incubated on media rich with lipoids (vitelline media), or on media which contain bile are insufficiently stable and cause a nonspecific agglomeration of alizarin suspension agglutinins and normal suspension globulins. The unfavorable influence of bile and lipoids on the stability of suspensions of microbial cells may be neutralized by adding to the nutrient media, 5-10% hemolyzed erythrocytes (a mixture of equal volumes of a precipitate of erythrocytes and distilled water). The application of normal suspension globulins makes it possible in all cases to reliably differentiate specific reactions from nonspecific.

In the case of investigation of microbial suspensions with the help of suspension antibodies, 3 variants of reactions are possible. In the first variant, the agglomeration is observed in a drop of suspension antibodies and is absent in a drop of normal suspension globulins.

The reaction is considered positive, if in the investigated suspension there are microbes homologous to the suspension antibodies. In the second variant, agglomeration in drops of suspension antibodies and normal suspension globulins is absent; the reaction is negative. In the third variant, agglomeration occurs in a drop of both suspension antibodies and also normal suspension globulins. In this case, if a pure culture is investigated, then the reaction is considered as negative; if the same reaction is set up with a suspension of a mixed culture, then the reaction is regarded as doubtful, since in mixed cultures the specific reaction may be veiled by nonspecific agglomeration of suspension globulins caused by saprophytic microbes. With the aim of elimination of nonspecific reactions, a method of elective sorption on trisubstituted calcium phosphate is used, after which the agglutination reaction is repeated.

#### Conclusions

1. The high sensitivity and specificity of suspension antibodies was caused by peculiarities in the structure of antibody--particle complexes, combining the properties of particles and immune antibodies.
2. Suspension antibodies are suited for the investigation of the nature of antigens and the identification of certain species of pathogenic microbes (causative agents of plague, botulism, and others) which cannot be identified with the aid of molecular antibodies. The employment of suspension antibodies opens wide possibilities for a more thorough study of the antigenic structure of microbes.



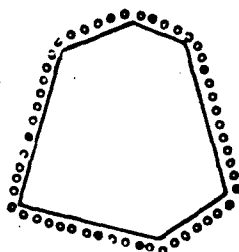


Figure 1. Schematic structure of suspension agglutinin.

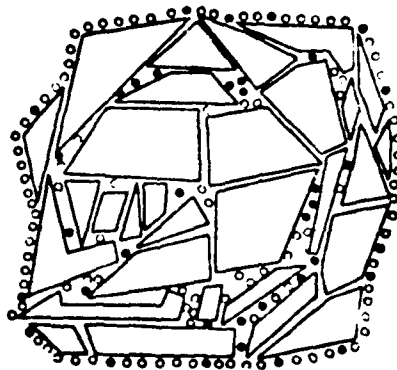


Figure 2. Structure of a suspension agglutinin made up of a conglomerate of alizarin particles.

Specificity of alizarin antiplague suspension agglutinins

Type of microbe	Number of strains	Agglutination reaction		Reaction of agglomeration of alizarin suspension antibodies	
		antiplague agglutinating serum	0.85% solution of sodium chloride	antiplague suspension antibodies	normal suspension globulins
P. pestis	20	3	3	20	-
P. pseudotuberculosis rodentium	17	2	-	--	-

Note. In the agglutination reaction sera was used, from which the suspension antibodies were prepared. The figures in the table denote the number of strains with which positive reactions were observed.

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